Impairment of Long-Term Potentiation in the CA1, But Not Dentate Gyrus, of the Hippocampus in Obese Zucker Rats

Role of Calcineurin and Phosphorylated CaMKII

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Abstract

Obese Zucker rat (OZR) is a genetic model of obesity with noninsulin-dependent diabetes and hypertension. The OZR exhibit hyperinsulinemia, hyperlipidemia, and high circulating glucocorticoid levels. We have shown previously that long-term potentiation (LTP) is impaired in the CA1 region of the hippocampus of OZR. In the present work, although electrophysiological recording from anesthetized OZR hippocampus showed impaired LTP in the CA1, an intact LTP was recorded in the dentate gyrus (DG) region of the hippocampus of the same OZR. Thus, LTP is differentially impaired in the CA1 compared with the DG region of OZR hippocampus. Immunoblotting was used to investigate the molecular mechanism responsible for impairment of LTP in the CA1 but not in the DG region. Analysis revealed reduction in the levels of phosphorylated calcium-dependent calmodulin kinase II (P-CaMKII) and total CaMKII in the CA1 region of OZR. However, in the DG region, reduction was observed only in the levels of total CaMKII, with no change in P-CaMKII levels. The ratio of P-CaMKII to total CaMKII was increased in the DG but not in the CA1 area of hippocampus of OZR. Although unchanged in the CA1, calcineurin levels were significantly reduced in the DG of OZR. These findings suggest that the DG might possess a compensatory mechanism whereby calcineurin levels are reduced to allow sufficient P-CaMKII to produce an apparently normal LTP in the DG area of OZR hippocampus.

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Index Entries: Immunoblotting; obese Zucker rats; electrophysiology.

Introduction

Calcium-dependent calmodulin kinase II (CaMKII) activity is necessary and sufficient to generate long-term potentiation (LTP) in the hippocampus (Malenka et al., 1989; Pettit et al., 1994; Thomas et al., 1994; Lledo et al., 1995; Giese et al., 1998; Roberts et al., 1998). A widely accepted cellular model for learning and memory (Bliss and Collingridge, 1993), LTP can be expressed both in vivo and in vitro. The molecular mechanism for the expression of LTP has been proposed to involve an array of signaling molecules. High-frequency stimulation (HFS) causes presynaptic release of glutamate, which activates NMDA receptors on the postsynaptic membrane thus elevating intracellular calcium levels (Malenka et al., 1988; Nicoll et al., 1989; Fukunaga, 1993). This highly localized, transient increase of intracellular calcium...
leads to the dissociation of the neurogranin-calmodulin complex (Gerendasy et al., 1994, 1995; Gerendasy and Sutcliffe, 1997; Krucker et al., 2002). The free calmodulin forms a calcium/calmodulin complex that binds to and activates CaMKII, triggering autophosphorylation (Wang and Kelly, 1995; Holmes, 2000). The phosphorylated CaMKII (P-CaMKII) activates substrates important in LTP expression, including synapsin-I, a vesicle-associated phosphoprotein, and the glutamate AMPA receptor (Fukunaga et al., 1996; Nayak et al., 1996; Barria et al., 1997a, 1997b). The activation of these substrates by P-CaMKII is uninterrupted, even when calcium returns to basal levels, until dephosphorylation of P-CaMKII by protein phosphatases, including calcineurin (Fukunaga et al., 1996; Wang and Kelly, 1996; Fukunaga and Miyamoto, 2000).

The Obese Zucker rat (OZR) is a genetic model of obesity with noninsulin-dependent diabetes and hypertension (Kasiske et al., 1992; Van Zwieten et al., 1996). Hypertension impairs memory in humans and in animal models (Wallace et al., 1985; Madden and Blumenthal, 1989; Levin et al., 1991; Meneses et al., 1996; Petrov et al., 1997). In addition, obesity is a risk factor for stress (Rosmond et al., 1998; Vincent et al., 1999; Perticone et al., 2001), which in itself could be responsible for the elevated blood pressure in OZR (Esler et al., 1977; Boone, 1991; Gerges et al., 2003a). Stress also impairs memory and LTP (Foy et al., 1987; Diamond et al., 1994; McEwen, 1999; Gerges et al., 2001). The OZR is stress prone, and, as with the obese man, it inadequately handles stress (Walker and Edwards, 1994; Suter et al., 1997). It has been shown that in the OZR, stress causes more than a sevenfold increase in corticosterone levels (Edwards et al., 2000). We have recently reported impaired LTP in the CA1 area of OZR hippocampus (Gerges et al., 2003a). In the present study, we show that although LTP is impaired in the CA1, it is spared in the dentate gyrus (DG) area of the hippocampus of the same OZR.

**Materials and Methods**

**Animals**

All animal experiments were carried out in accordance with NIH guidelines for care and use of laboratory animals and approved by the University of Houston’s Institutional Animal Care and Use Committee. Experiments were done in adult male OZRs and their genetic control, the lean Zucker rats (LZRs) (Harlan Sprague-Dawley, Indianapolis, IN), aged 19–20 wk.

**Western Blotting**

Animals were sacrificed, and the CA1 and DG regions of the hippocampus were immediately dissected out and homogenized individually in 200 μL of buffered isotonic cocktail containing protease and phosphatase inhibitors (150 mM NaCl, 0.075 mM pepstatin, 0.1 mM leupeptin, 1 mM PMSF, 5 mM benzamidine, 1 mM EDTA, 1 mM EGTA, 20 mM Tris, 15 mM Na3P2O7, 100 mM B-glycerophosphate, 25 mM NaF). The samples were sonicated, and total protein was estimated by BCA assay (Pierce Chemical, Rockford, IL). Samples were stored at −80°C until use. Just before use, samples were diluted (using...